Electron irradiation effects on nanocrystal quantum dots used in bio-sensing applications

R. Leon[1], J. Nadeau[1], K. Evans[1], T. Paskova[2], and B. Monemar[2]

[1] *Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, CA 91109*

[2] *Department of Physics and Measurement Technology, Linköping University, S-581 83, Linköping, Sweden*

Effects of electron irradiation on some of the optical properties inorganic CdSe nanocrystals coated in trioctylphosphine oxide (TOPO) and biologically compatible CdSe nanocrystals coated in mercaptoacetic acid, as well as CdSe nanocrystals conjugated with the protein are investigated using the technique of cathodoluminescence. Effects of varying the beam energy and temperatures were examined and faster degradation at cryogenic temperatures and higher beam energies was found under some conditions.

INTRODUCTION

Colloidal quantum dots (QDs), also known as nanocrystals, are synthesized by chemical means and dispersed in suitable solutions. Like all QDs, nanocrystals owe their unique optical properties to the effects of three-dimensional quantum confinement; hence, their emission wavelengths increase with particle dimensions, and they can be tuned to emit at different wavelengths by controlling their sizes [1]. Nanocrystals have several proven and potential applications as biosensors. In one sensing application, QDs are used in lieu of fluorescent dyes [2-5], with the advantage of greater stability for long missions, and the capability of inducing multi-color fluorescence with a single excitation wavelength. QDs have also recently shown promise as reducing oxidizing sensors (redox), by acting as on-off biological sensors that fluoresce only in the presence of living organisms.

Fluorescence is one of the most sensitive and specific methods for labeling microorganisms and fragments of microorganisms [6] It remains the tool of choice for "life detection" experiments on earth, where samples from extreme environments are examined for traces of living or fossilized life [7]. Fluorescent dyes may be added directly to samples of soil, rock, water, or ice; some of these dyes fluoresce only in the presence of specific targets such as lipid membranes or DNA.

The use of this technique for Martian *in situ* instruments is feasible and is currently being proposed by multiple investigators. Miniaturized microscopes and fluorimeters exist which could fit into a Rover instrument package and which theoretically have detection limits as low as a single fluorescently-labeled bacterial cell.

The lack of dyes with suitable properties for space flight remains the biggest obstacle. Sterilization procedures for planetary protection will destroy most organic dyes. Radiation bombardment, freeze-thaw, and other rigors of space flight also lead to fluorescence loss. If biologically compatible quantum dots are shown to endure these conditions more readily than existing dyes, they could serve as a viable alternative. In addition to being more resistant, quantum dots provide other advantages: they yield many output wavelengths for a single emission wavelength, permitting the use of a single miniature laser as an excitation source. This is an enormous advantage for space applications as it greatly reduces the mass and power requirements. The output spectra of QDs are narrow, so that a single sample may be labeled with

4-5 probes, again of critical importance to in situ applications where samples are precious and washing is impossible.

Streptavidin is one of the most useful QD conjugates because it serves to stabilize the particle as well as to create a "bridge" between the QD and any biomolecule of choice [8]. Streptavidin possess 4 binding sites for the small molecule biotin; the binding is the strongest non-covalent linkage known. Thus, any molecule may be attached to biotin and then readily conjugated to streptavidin in a manner that is extremely stable and strong and whose stoichiometry is known. The only times QDs are used in biological applications without streptavidin coats is when the protein would interfere with processes such as Förster energy transfer [9, 10].

These sensors will be more useful in space applications if they demonstrate certain tolerance to extremes in temperature, radiation, and oxidation conditions. The robustness of these nanocrystals needs to be established before they can fulfill their promise as biological probes for space applications. Recent efforts focusing on epitaxial Stranski-Krastanow QDs have demonstrated superior radiation resistance due to the effects of three-dimensional quantum confinement, even showing an increase in the luminescence intensity [11, 12] or diode laser performance [13] after considerable proton fluences. Hence, greater tolerance to space radiation could be another potential advantage of using nanocrystals as biosensors in space application.

Here we use CL spectroscopy to examine the effects of electron irradiation on the optical properties of both inorganic and biologically compatible CdSe nanocrystal quantum dots.

In semiconductor characterization, cathodoluminescence (CL) is a powerful tool for defect recognition and imaging of lattice defects. The spatial distribution of impurities and radiation-induced defects can be imaged with high resolution. CL can give valuable information on performance degradation due to non-radiative recombination and can determine the spatial distribution of deep level centers in semiconductors microelectronic materials and devices.

Another application of CL is in electron radiation damage studies, since an energetic electron beam may induce defects in the specimen. CL microanalysis allows the formation of irradiation-induced defects to be monitored and investigated. Since the optical properties of some materials and devices can be characterized with CL spectroscopy, this gives the advantage that a whole irradiation experiment, including monitoring of the degradation in optical properties and identification of localized damage can be done in *in situ*.

EXPERIMENTAL DETAILS

In this work, results are compared for CdSe QDs coated with trioctylphosphine oxide (TOPO), and QDs coated with mercaptoacetic acid (MAA). Nanocrystals coated with MAA and conjugated with proteins (streptavidin) are also examined. Published results on nanocrystal characterization have been obtained from either CdSe or CdSe/ZnS QDs in TOPO. Effects of TOPO include passivation of the surface of the QDs, reducing surface states and enhancing the luminescence emission. However, these colloidal nanoparticles are only soluble in non-polar solvents. Substitution of the TOPO coating with MAA allows solubilization in polar solvents, like water, and it also allows binding organic molecules to the external surface of the QDs. MAA hence is essential in making these quantum dots biologically compatible.

Alternative methods of solubilization: MAA or closely related compounds (mercaptopropionic acid or mercaptoundecanoic acid) remain the most common methods for solubilizing QDs. However, other methods are also used, some of which have proven more stable. Bidentate thiols such as dihydrolipoic acid can be used to create a stronger QD-cap bond [14], or QDs may be coated with Si shells [2] or dendrimers [15].

Details of the synthesis can be found elsewhere [16]. Since NaCl can give CL signals, these samples were additionally processed to remove the salt when the nanocrystals were dispersed in buffered saline solutions by centrifuging the particles after conjugation and washing with distilled water. All samples were purified after conjugation by centrifugation and/or dialysis, so there is no unconjugated protein left in the sample.

For bare CdSe, green, emission peak 575 nm, mean diameter ± SD 3.9 ± 0.4 nm; yellow, emission peak 587 nm, mean diameter 4.6 ± 0.4 nm; and red, emission peak 615 nm; mean diameter 5.4 ± 0.4 nm. For core-shell CdSe/ZnS QDs, green, emission peak 565 nm, mean diameter 3.6 ± 0.3 nm; red, emission peak 620 nm, mean diameter 6.5 ± 0.5 nm.

Cathodoluminescence (CL) imaging and spectroscopy were carried out using a scanning electron microscope equipped with a monochromator attachment for CL spectroscopy and a GaAs photo multiplier detector sensitive in the visible range (300 to 800 nm). CL was performed on nanocrystal samples that were dried into microscope aluminum sample holders. Some of the samples were illuminated prior to drying in order to evaluate how oxide formation around the CdSe quantum dots affects their CL emission.

Images and spectra were collected either at room temperature or at 5 K, and various accelerating voltages, beam apertures, and magnifications were used in order to vary both the irradiation energy and dose rates.

Results:

The cathodoluminescence signal intensity is seen to diminish with successive spectral acquisitions, showing that electron irradiation, even in the KeV range, can cause damage and affect the optical properties of CdSe nanocrystals.

Figure 1 shows the cathodoluminescence spectra and a collection of successive spectral acquisitions of the same samples with electron beam irradiation. The broad emission from CdSe nanocrystals originates from slight variations in the nanocrystal sizes, giving a roughly Gaussian distribution in their emission spectra. The closer the size calibration, the narrower the luminescence emission spectra is from these QD.

Nanocrystal emission in Fig 1 (b) is at the longer wavelengths, the shorter wavelength peak apparent in these spectra originates from incomplete removal of the sodium chloride in the buffered saline solutions where the MAA and bio conjugated nanocrystals were suspended.

The small signal apparent from Fig.1 (c) is directly related to optical quenching effects from the MAA coating. The luminescence can be made to increase to some extent upon illumination in the presence of oxygen, but in conjugated nanocrystals oxidation surface is unavailable and the signal remains weak.

CL emission degradation with electron dose was quantified by measuring the electron beam current, recording the irradiated area and timing the exposure to the electron beam. After such analysis, intensity degradation plots as shown in Fig. 2 can be obtained. In order to eliminate potential charging effects that might have an effect on CL intensities, the beam was turned off for several hours and the CL spectra was recorded again. No significant recovery of the CL intensity was observed.

The emission from CdSe nanocrystals with bio and non-bio compatible coatings and CdSe nanocrystals conjugated with organic molecules were compared using both room temperature and cryogenic temperatures. Different electron energies were also used in these experiments.

Figure 2 shows the effects of electron irradiation at cryogenic temperatures (4.6 K) vs room temperature irradiation under the same conditions. MAA and STREP conjugated nanocrystals show increased sensitivity to electron beam effects at low temperatures, and inorganic TOPO coated nanocrystals who increased sensitivities for low beam energies. At higher beam energies (30 keV) the effects of cryogenic irradiation were inconclusive.

Figure 3 shows a similar comparison, but taking as a variable the electron beam energy (room temperature measurements). Here we see that for both TOPO and MAA coated nanocrystals the damage saturates at lower fluences and is overall less for lower e beam energy at the same fluences. A notable difference was seen in the protein conjugated nanocrystals, where less damage was seen after 30 keV irradiations. This observation also explains the enhancement of electron beam effects on the luminescence emission shown in Figure 4 (b) as compared with Fig 4 (a).

Figure 4 shows that under some conditions, effects of electron irradiation is equivalent in bio compatible, conjugated and inorganic nanocrystals, however, difference can arise under lower beam energies, as seen in the differences between the 30 keV and 5 keV experiments.

Discussion:

CL of biologically compatible QDs has not been reported. However, there have been numerous CL studies using CdSe QDs grown by MBE, at both room temperature and cryogenic temperatures, resulting in visualization of excitons and biexcitons [17, 18]

These results show not only that CL can be used to monitor electron induced degradation, but that it can be a valuable tool investigating environmental effects, and in understanding how electron radiation effects can change at cryogenic temperatures. The differences observed in Fig. 4 in the relative effects of electron beam irradiation on the semiconductor quantum dots are probably related to the lack of thermal annealing of Frenkel pairs under cryogenic conditions, which would allow damage to accumulate more readily in the semiconductor and would not have such significant effect on the organic component of the sample.

While electron irradiation has been shown to alter the properties of many semiconductor materials, this rapid degradation can be surprising in light or earlier results [11, 12] that showed epitaxial quantum dots to be orders of magnitude more radiation tolerant to proton induced damage than quantum wells from the same materials family. Two factors must be considered to understand these results: (i) the damage threshold in II-VI materials is known to be considerable lower than in other commonly used semiconductor materials [19], and (ii) the radiation conditions and geometries in these nanocrystal QDs are completely different since the nanocrystals in this present study are bare, without the benefit of a large volume of large bandgap semiconductor cladding or surrounding the quantum dots.

CONCLUSIONS

We have shown that the technique of cathodoluminescence can be successfully utilized as a quantitative tool for *in-situ* monitoring of electron damage in materials. This technique is applied to the study of nanocrystal quantum dots with inorganic passivation, bio-compatible passivation and conjugated with organic molecules. Our results show significant degradation in the luminescence emission from nanocrystals after electron beam exposure in the KeV ranges, and faster degradation from nanocrystal luminescence emission when exposed to the electron beam at cryogenic temperatures.

REFERENCES

[1]. "(CdSe)ZnS Core-Shell quantum dots: synthesis and characterization of a size series of highly luminescent nanocrystallites", B. O. Dabbousi et al., J. Phys. Chem. B Vol. 101, p. 9463 (1997).

[2]. "Semiconductor nanocrystals as fluorescent biological labels", M. Bruchez, M. Moronne, P. Gin, S. Weiss, and A. P. Alivisatos, Science 281, 2013 (1998).

[3]. "Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules", M. Han, X. Gao, J. Z. Su, and S. Nie, *Nat Biotechnol* 2001, *19*, 631.

[4]. "Long-term multiple color imaging of live cells using quantum dot bioconjugates", J. K. Jaiswal, H. Mattoussi, J. M. Mauro, S. M. Simon, *Nat Biotechnol* 21, 47 (2003).

[5]. "Observation of solvatochromism in CdSe colloidal quantum dots" C. A. Leatherdale, M. G. Bawendi, *Physical Review B, 6316* (2001).

[6]. "Fluorescent probes applied to physiological characterization of bacterial biofilms" J. T. Lisle, P. S. Stewart, and G. A. McFeters, *Methods Enzymol* 310, 166 (1999).

[7]. "Development of detection system for extraterrestrial microorganisms", Y. Kawasaki, *Biol. Sci. Space* 8, 103 (1994).

[8]. "Avidin: A natural bridge for quantum dot-antibody conjugates", E.R. Goldman, E. D. Balighian, H. Mattoussi, M. K. Kuno, J. M. Mauro, P. T. Tran, G. P. Anderson, *Journal of the American Chemical Society* **124**, *6378* (2002).

[9]. "FRET between CdSe quantum dots in lipid vesicles and water- and lipid-soluble dyes", J. A. Kloepfer, N. Cohen, and J. L. Nadeau, Journal of Physical Chemistry B, in press

[10]. "Self-assembled nanoscale biosensors based on quantum dot FRET donors", I. L. Medintz, A. R. Clapp, H. Mattoussi, E. R. Goldman, B. Fisher, J. M. Mauro, *Nature Materials* **2**, 630 (2003).

[11]. "Changes in Luminescence Emission Induced by Proton Irradiation: InGaAs/GaAs Quantum Wells and Quantum Dots", R. Leon, G. M. Swift, B. Magness, W. A. Taylor, Y. S. Tang, K. L. Wang, P. Dowd, and Y. H. Zhang, Appl. Phys. Lett. 76, 2071 (2000)

[12] Effects of proton irradiation on luminescence emission and carrier dynamics of self-assembled III-V quantum dots", Leon R, Marcinkevicius S, Siegert J, Cechavicius B, Magness B, Taylor W, Lobo C, IEEE Trans. Nucl. Sci. **49**, 2844 (2002).

[13]. "Enhanced degradation resistance of quantum dot lasers to radiation damage", P. G. Piva, R. D. Goldberg, I. V. Mitchell, D. Labrie, R. Leon, S. Charbonneau, Z. R. Wasilewski, and S. Fafard, Appl. Phys Lett. 77, 624 (2000)

[14]. "Self-assembly of CdSe-ZnS quantum dot bioconjugates using an engineered recombinant protein" H. Mattoussi, J. M. Mauro, E. R. Goldman, G. P. Anderson, V. C. Sundar, F. V. Mikulec, M. G. Bawendi, Jour. Am. Chem. Soc. 122, 12141 (2000)

[15] "Comparison and stability of CdSe nanocrystals covered with amphiphilic poly(amidoamine) dendrimers", C. X. Zhang, S. O'Brien, L. Balogh, J. Phys. Chem. B 106, 10316 (2002)

[16] "Quantum dots as strain- and metabolism-specific microbiological labels", J.A. Kloepfer, R.E. Mielke, M. S. Wong, K. H. Nealson, G. Stucky, and J. L. Nadeau, Appl. and Envir. Microbiology, 69, 4205 (2003)

[17]. "E-beam irradiation effect on CdSe/ZnSe QD formation by MBE: deep level transient spectroscopy and cathodoluminescence studies", V. I. Kozlovsky, V. G. Litvinov, and Yu. G. Sadofyev, J. Phys. C 16, S133 (2004).

[18]. "Cathodoluminescence and photoluminescence decay behaviors of CdSe dots embedded in ZnSe", I. Yamakawa, M. Ichida, S. V. Sorokin, A. A. Toropov, A. N. Titkov, S. V. Ivanov, and A. Nakamura, J. of Luminescence 87, 384 (2000).

[19]. "Large band-gap SiC, III-V nitride, and II-VI ZnSe-based semiconductor device technologies", H. Morkoç, S. Strite, G. B. Gao, M. E. Lin, B. Sverdlov, and M. Burns, J. Appl. Phys. 76, 1363 (1994).

Figure Captions:

Figure 1. (a) Cathodoluminescence spectra for CdSe quantum dot nanocrystals coated in TOPO. Curve set shows successive scans taken at the same spot, showing the effect of electron beam damage on the cathodoluminescence intensity. (b) Successive cathodoluminescence scans of CdSe nanocrystals coated in mercapto acetic acid (MAA) taken of the same location in the sample. The low wavelength (high energy) peak at around 400 nm originates from residual sodium chloride in the sample. (c) Spectra and successive CL scans of the same sample showing degradation in CdSe nanocrystals coated in MAA and conjugated with Streptadivin. Weaker emission from this sample is apparent from the noisier spectra.

Fig 2 (a) Comparison of 30 keV electron irradiation induced degradation at room temperature and 4.6 K for CdSe nanocrystals conjugated with streptadivin. (b) Comparison of electron irradiation induced degradation at 5 keV beam energy at room temperature and 4.6 K for CdSe nanocrystals coated in TOPO.

Fig 3 (a) Comparison of degradation in CdSe nanocrystals in MAA under different beam energies (10 and 30 keV) at room temperature. (b) Comparison of degradation in CdSe nanocrystals in TOPO under different beam energies (5, 15 and 30 keV) at room temperature. (c) Comparison of degradation in CL intensity for CdSe nanocrystals conjugated with streptadivin under different beam energies (5, 10 and 30 keV).

Fig 4 (a) Comparison of 30 keV electron beam induced degradation on the inorganic nanocrystals (CdSe in TOPO) the biologically compatible 'bare' nanocrystals (CdSe in MAA) and the Streptadivin protein conjugated CdSe nanocrystals. (b) Comparison of 5keV electron beam induced degradation on the inorganic nanocrystals (CdSe in TOPO) the biologically compatible 'bare' nanocrystals (CdSe in MAA) and the Streptadivin protein conjugated CdSe nanocrystals. *Merge these two*

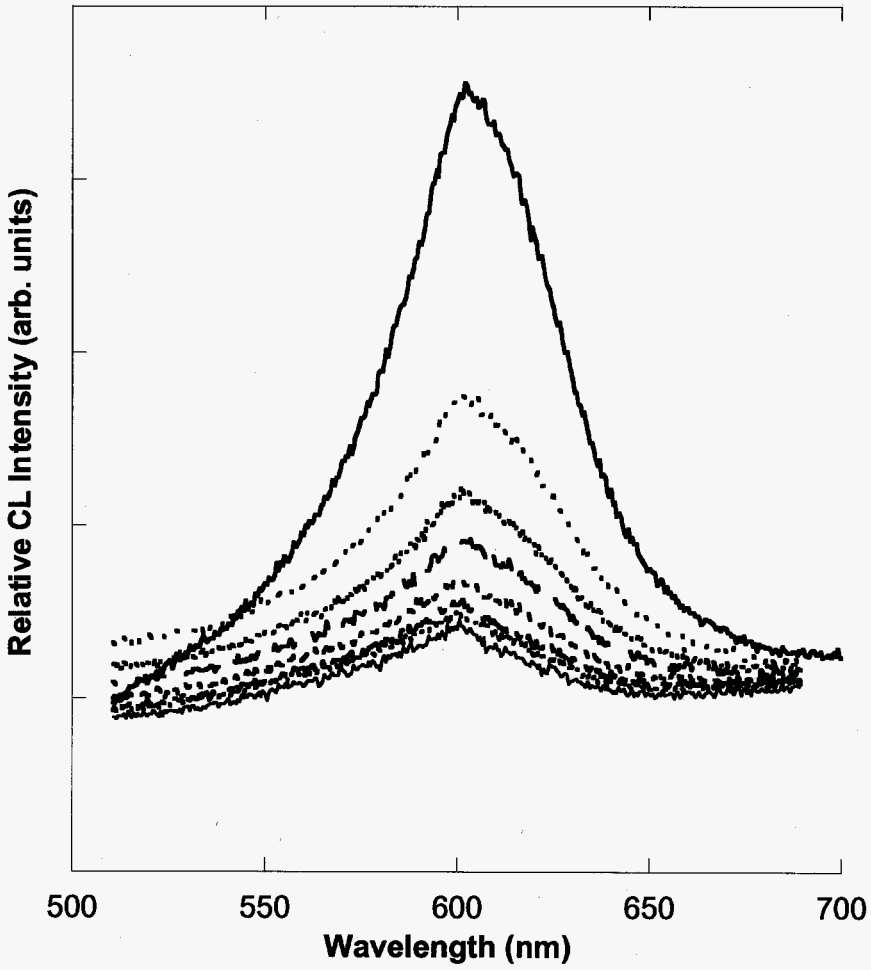

Figure 1 (a), R. Leon

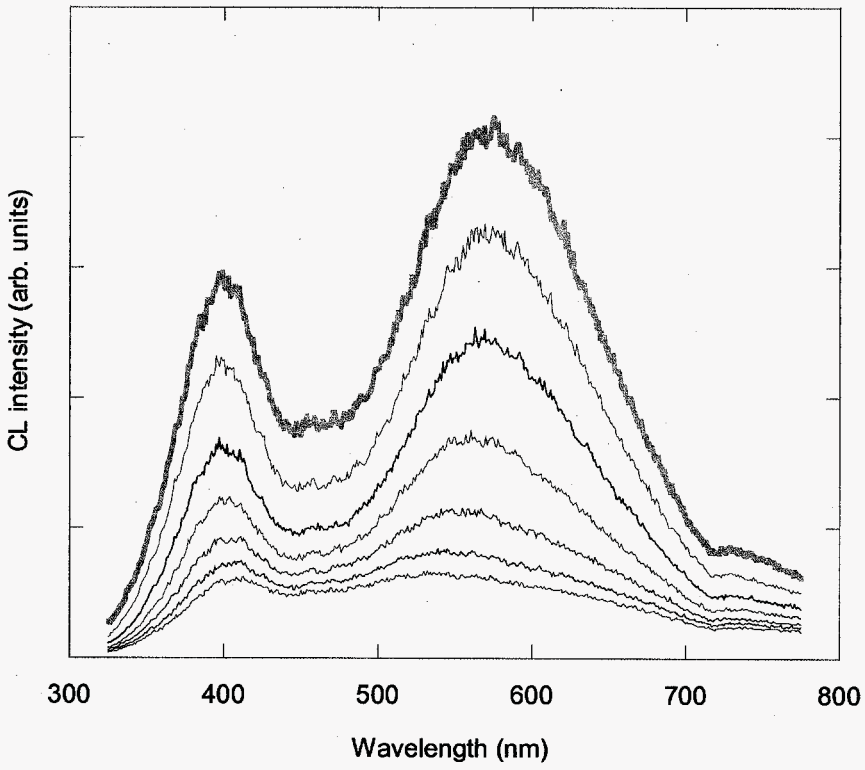

Figure 1 (b), R. Leon

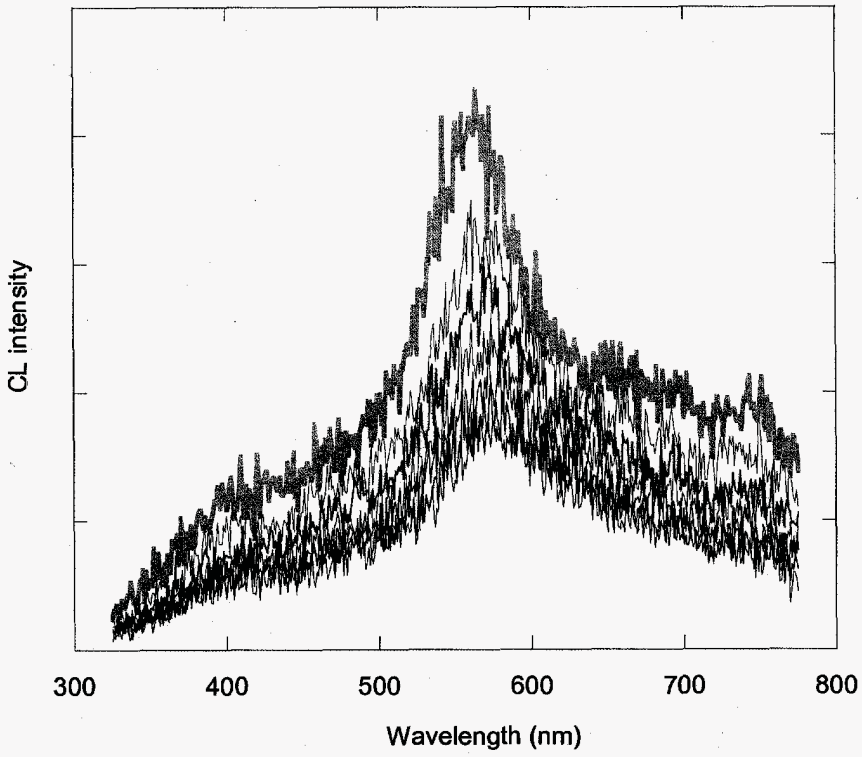

Figure 1 (c), R. Leon

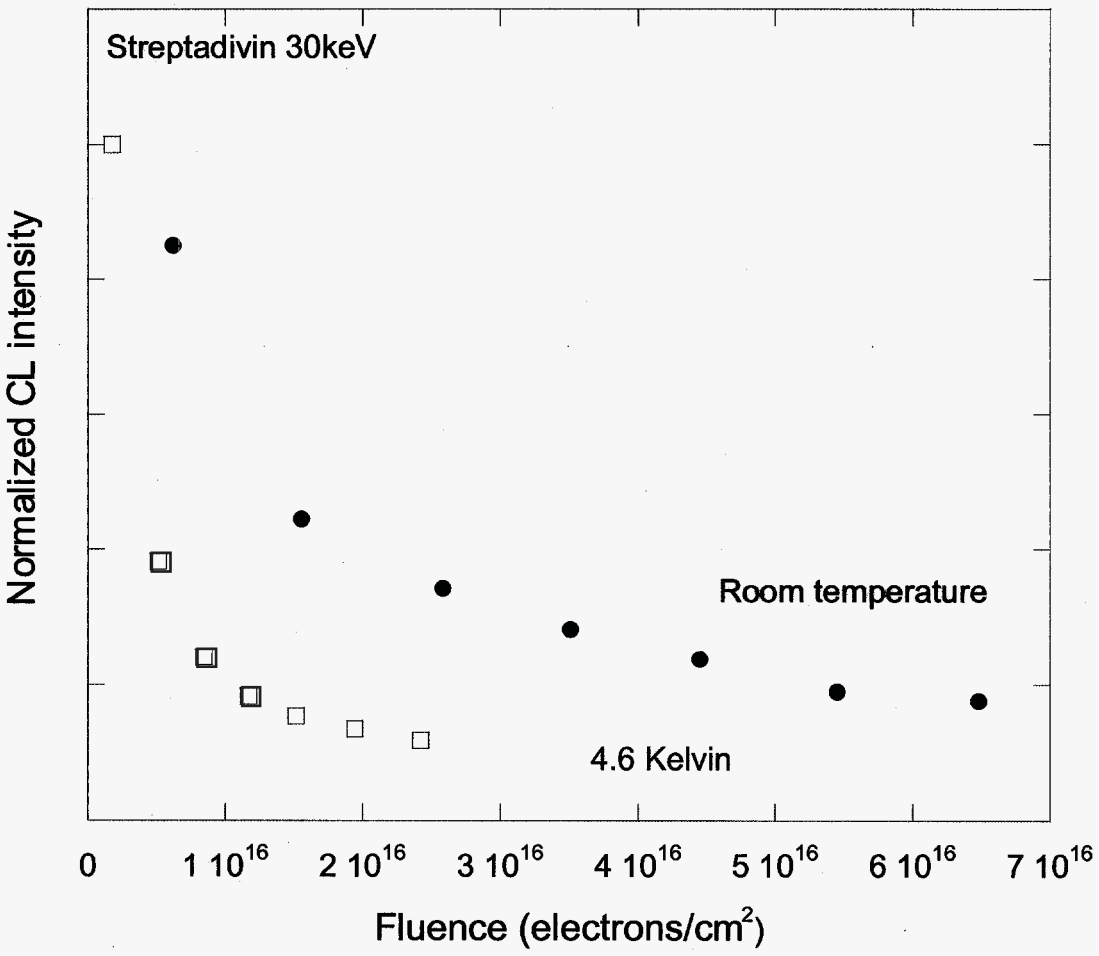

Figure 2 (a), R. Leon

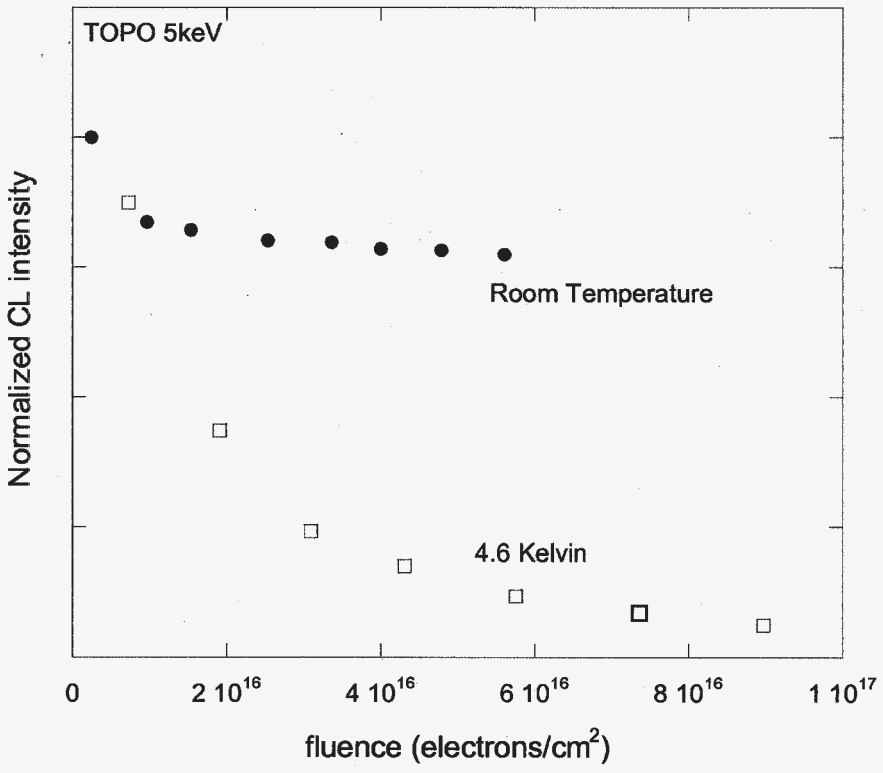

Figure 2 (b), R. Leon

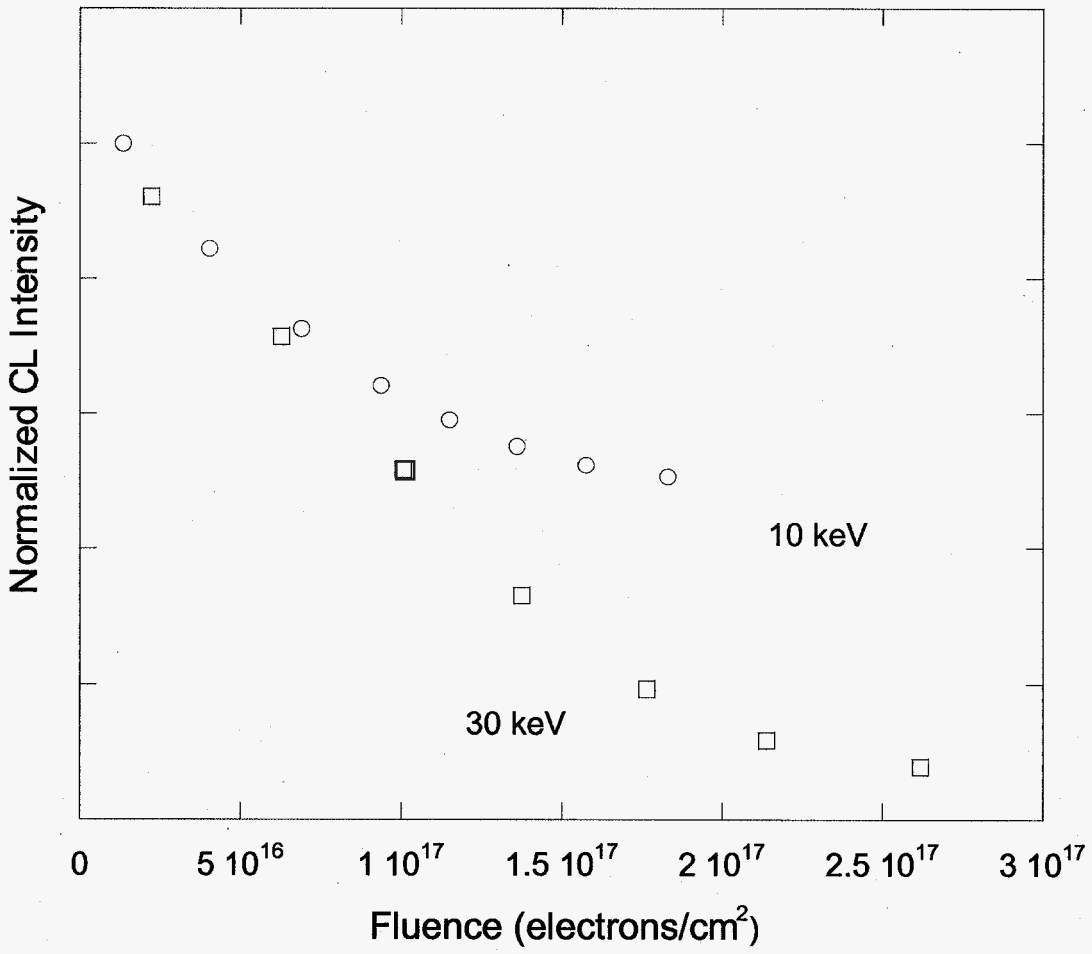

Figure 3 (a), R. Leon

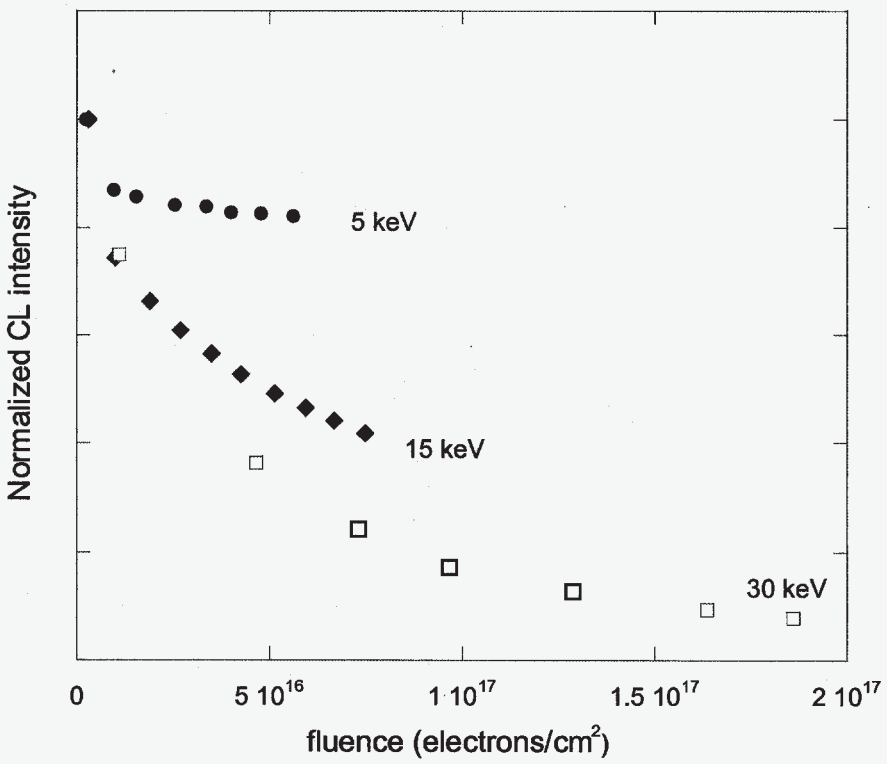

Figure 3 (b), R. Leon

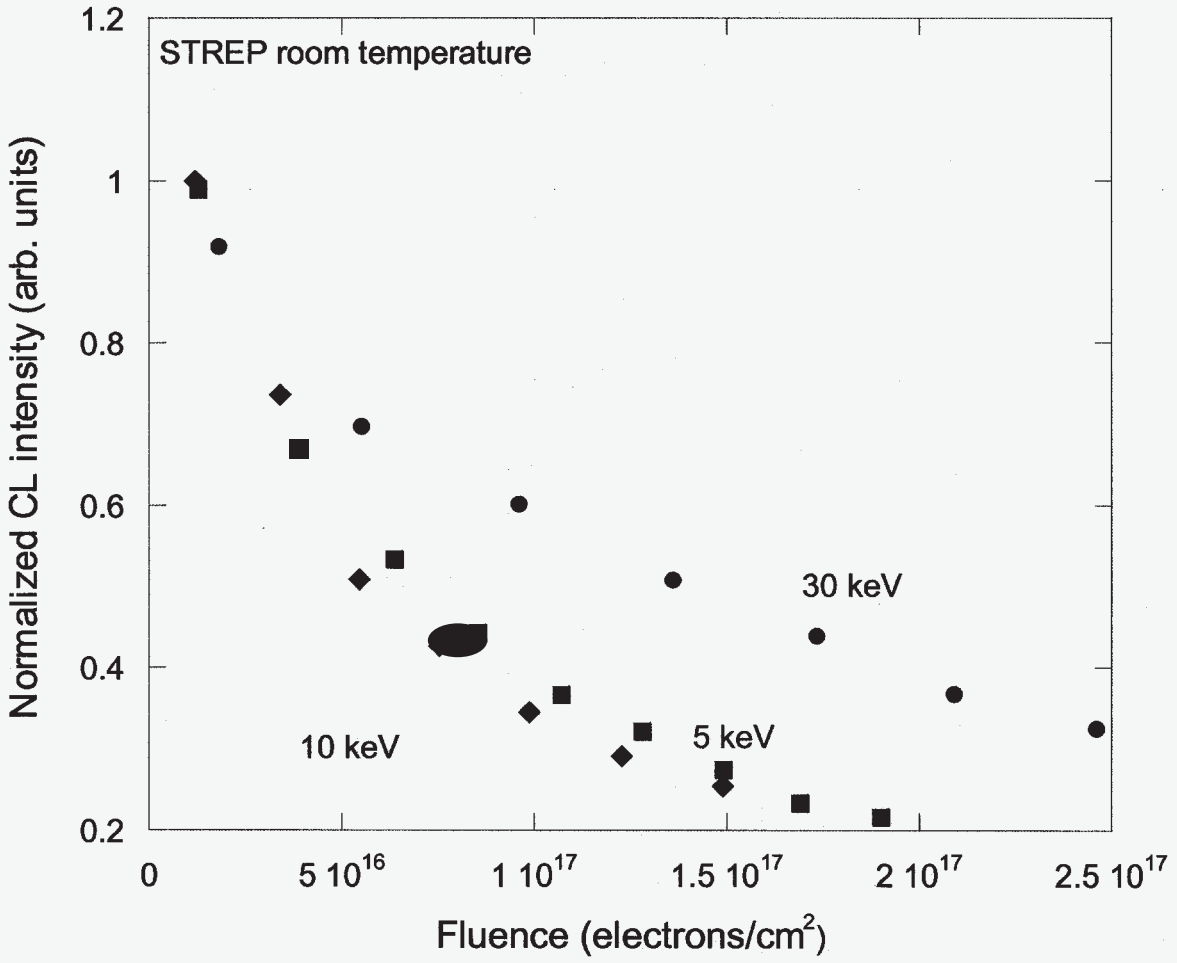

Figure 3 (c), R. Leon

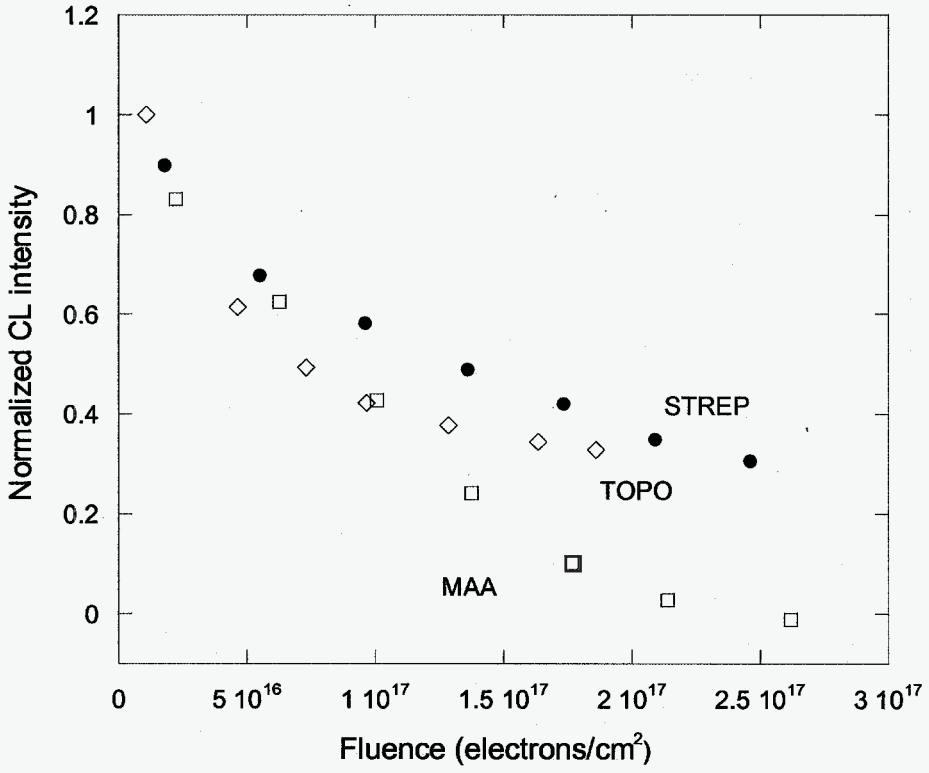

Figure 4 (a), R. Leon

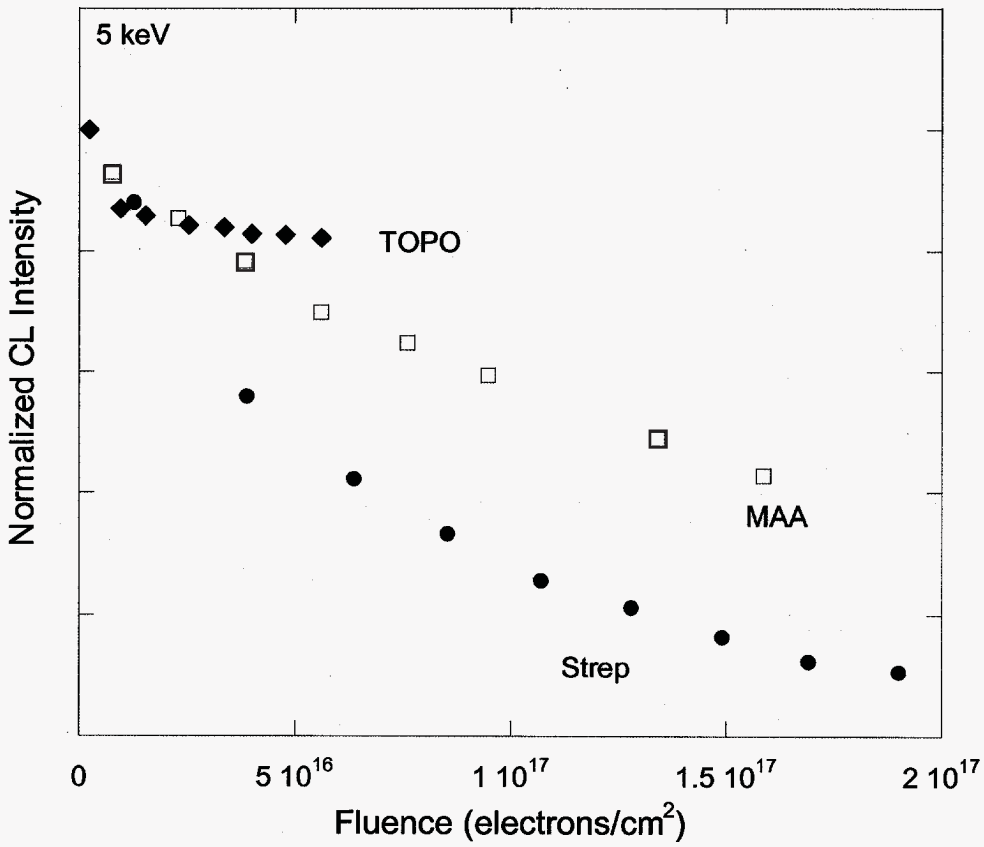

Figure 4 (b), R. Leon

End of File